An impedimetric immunosensor based on diamond nanowires decorated with nickel nanoparticles

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Nanostructured boron-doped diamond has been investigated as a sensitive impedimetric electrode for the detection of immunoglobulin G (IgG). The immunosensor was constructed in a three-step process: (i) reactive ion etching of flat boron-doped diamond (BDD) interfaces to synthesize BDD nanowires (BDD NWs), (ii) electrochemical deposition of nickel nanoparticles (Ni NPs) on the BDD NWs, and (iii) immobilization of biotin-tagged anti-IgG onto the Ni NPs. Electrochemical impedance spectroscopy (EIS) was used to follow the binding of IgG at different concentrations without the use of any additional label. A detection limit of 0.3 ng mL⁻¹ (2 nM) with a dynamic range up to 300 ng mL⁻¹ (2 μM) was obtained with the interface. Moreover, the study demonstrated that this immunosensor exhibits good stability over time and allows regeneration by incubation in ethylenediaminetetraacetic acid (EDTA) aqueous solution.

1. Introduction

Imunoassays relying on antibody–antigen interactions provide a promising means of analysis due to their specificity and sensitivity. High specificity is achieved mainly by the molecular recognition of the target analyte by antibodies or antigens through the formation of a stable immunocomplex. The binding affinities are typically in the range of $K_a = 10^{-3}$ to $10^{-7}$ M and are dictated by the type of interaction involved and the surface area buried in the paratope–epitope interface. Immunosensing has typically relied on standard sandwich bioaffinity assays in conjunction with labels such as enzymes, fluorophores or nanoparticles. Electrochemical immunosensing based on electrochemical impedance spectroscopy (EIS) has become a widely accepted alternative method for the detection of antigen–antibody interactions. By measuring the frequency dependence of the electrical impedance, EIS provides information that can be used to disentangle the complexes. This label-free real time analytical method has shown to be capable of providing equivalent analytical characteristics in terms of selectivity and reliability with the additional advantages of being easy to implement, fast and cost effective. Furthermore, compared to immunosensors using labels, electrochemical sensors offer higher detection limits. Recently Kim and co-workers reported on ultrasensitive antigen detection with a limit of detection of 100 fg mL⁻¹ using a reduced graphene oxide-based electrochemical immunosensor with horseradish peroxidase labeled secondary antibodies as amplifiers. An alkaline phosphatase (ALP) amperometric immunosensor with a detection limit of 0.3 ng mL⁻¹ has been developed by Einaga and it is based on the use of boron-doped diamond (BDD) electrodes modified with poly-(ε-aminobenzoic acid). The outstanding properties of BDD interfaces have been suggested to be responsible for the high sensitivity. Indeed, comparable experiments performed on glassy carbon (GC) electrodes resulted in a detection limit of 3.5 ng mL⁻¹. Lately, we and others have demonstrated that nanostructured BDD electrodes have considerable advantages over flat BDD interfaces in terms of sensitivity and selectivity. They combine the unique features of smooth BDD interfaces such as high chemical stability, low background current, wide potential window, decreased biofouling and high biocompatibility, with an increased surface area. We have also shown that the length of the wires influences the sensitivity of the interface. Motivated by this finding, we investigated here the potential application of long BDD nanowires (BDD NWs) as substrates for impedimetric immunosensors using immunoglobulin G (IgG) as a model analyte. IgG, a 150 kDa monomer constituting approximately 75% of the total circulating immunoglobulin, plays a crucial role in the immune system and is the main base of any immunosensor used for diagnostics. In this study, anti-bovine IgG was used as a model antibody to be immobilized onto the BDD NWs. Optimal immobilization of antibody molecules...
depends on the surface employed as well as on the respective surface tethering approach. Nanoparticle modified BDD NWs have shown to be highly appealing for electroanalytical applications as they show improved catalytic activity due to the high surface area, which in turn necessitates the use of much less material. An additional advantage is the enhanced diffusion observed at nanoparticles due to convergent rather than linear diffusion at slow scan rates at small particles.

In this context, BDD NWs coated with nickel nanoparticles (BDD NWs/Ni NPs), deposited electrochemically, are investigated as immobilization platforms for biotin-labeled IgG (Fig. 1A).

2. Experimental

2.1. Materials

Potassium hydroxide, nickel(II) sulfate heptahydrate (NiSO₄·7H₂O, 99.999%), phosphate buffer saline tablets (PBS, pH 7.4), bovine serum albumin (BSA), ethylenediaminetetraacetic acid (EDTA), Fe(CN)₆³⁻/⁴⁻ and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich and used without purification. Biotin conjugated rabbit anti-bovine IgG (H&L) and rabbit bovine IgG were obtained from Agrisera Antibodies, Sweden.

2.2. Preparation of boron-doped diamond nanowires (BDD NWs) decorated with nickel nanoparticles (BDD NWs/Ni NPs)

Boron-doped diamond films were grown by microwave plasma-enhanced chemical vapor deposition from methane-hydrogen mixtures (1% CH₄) in an ASTeX 6500 reactor. The substrates were p-type doped (100) silicon wafers (thickness 50–500 μm, resistivity from 1 to 20 Ω cm). Trimethyl borane gas was added during the growth with a ratio of 10 000 ppm B/C to CH₄ to ensure good electrical conductivity. The final film thickness was 15 μm and the boron concentration was N_A = 8 × 10¹⁹ B cm⁻² according to SIMS measurements.

Boron-doped diamond nanowires (BDD NWs) were prepared using reactive ion etching (RIE) of BDD using an oxygen plasma (Plasmalab 80plus) with a radiofrequency (RF) generator (13.56 MHz) for 180 min. The operating oxygen pressure, flow speed, and plasma power were 150 mT, 20 sccm, and 350 W, respectively. The resulting BDD NWs were immersed for 15 min in an aqueous solution of HF (5% v/v) to dissolve the SiO₂ deposited on the wires during the etching process.

Nickel nanoparticles (Ni NPs) were deposited onto BDD NWs by reduction of an aqueous solution of NiSO₄ (5 mM) in PBS buffer (0.01 M; pH 6.5) at −1.3 V for 200 s under moderate stirring, at 0 V for 1 min followed by another pulse at −1.3 V for 200 s. This cycle was repeated 10 times. After deposition, the modified electrode was immersed in NaOH (0.1 M) and cycled 300 times between 0 and 0.5 V to enrich the Ni(OH)₂ deposit.

2.3. Binding of biotin-tagged anti-IgG onto BDD NWs/Ni NPs

The BDD NW/Ni NP interface was immersed for 100 min into biotin-labeled anti-IgG (0–100 μg mL⁻¹) in PBS buffer (0.1 M; pH 7.4) and then thoroughly washed three times under sonication with PBS. To limit the non-specific interaction of IgG with the surface, the BDD NW/Ni NP-anti IgG modified interface was furthermore immersed for 100 min into an aqueous bovine serum albumin (BSA, 100 μg mL⁻¹ PBS solution) and thereafter thoroughly washed three times under sonication with PBS.
After interaction with IgG, the antibody–antigen complex was dissociated by incubation of the interface in acidic glycine buffer (pH = 3) for 30 min under sonication followed by washing (three times) with PBS.

Bonded anti-IgG could be washed off the BDD NW/Ni NP interface by incubation in an aqueous solution of ethylenediaminetetraacetic acid (EDTA 0.01 M) for 60 min under sonication followed by washing (three times) with PBS.

When not in use the immunosensor was stored in PBS (0.01 M, pH 7.4) at 4 °C.

2.4. Characterization

2.4.1. Scanning electron microscopy (SEM). SEM images were obtained using an electron microscope ULTRA 55 (Zeiss) equipped with a thermal field emission emitter and a high efficiency In-lens, and ESB and SE detectors.

2.4.2. X-ray photoelectron spectroscopy (XPS). X-ray photoelectron spectroscopy (XPS) experiments were performed using a PHI 5000 VersaProbe-Scanning ESCA Microprobe (ULVAC-PHI, Japan/USA) instrument at a base pressure below 5 × 10⁻⁹ mbar. Monochromatic AlKα radiation was used and the X-ray beam, focused on a diameter of 100 μm, was scanned on a 250 × 250 μm surface, at an operating power of 25 W (15 kV). Photoelectron survey spectra were acquired using a hemispherical analyzer at a pass energy of 117.4 eV with a 0.4 eV energy step. Core-level spectra were acquired at a pass energy of 23.5 eV with a 0.1 eV energy step. All spectra were acquired with sensitivity factors supplied by PHI.

2.4.3. Cyclic voltammetry. Cyclic voltammetry (CV) experiments were performed using an Autolab 20 potentiostat (Eco Chimie, Utrecht, The Netherlands). The electrochemical cell consisted of a working electrode (BDD NWs/Ni NPs), Ag/AgCl (Bioanalytical Systems, Inc.) as a reference electrode, and platinum wire as a counter electrode. Cyclic voltammetric measurements were performed at a scan rate v = 0.05 V s⁻¹.

2.4.4. Electrochemical impedance spectroscopy (EIS). Electrochemical impedance measurements were performed using an Autolab 20 potentiostat (Eco Chimie, Utrecht, The Netherlands). EIS experiments were carried out in an aqueous solution of a mixture of 10 mM Fe(CN)⁶⁴⁻/10 mM Fe(CN)⁶³⁻ in PBS (0.01 M) using the following parameters: amplitude of 10 mV at an open circuit potential with a frequency range of 100 kHz–0.1 Hz. Impedance data were modeled using ZView2 software.

3. Results and discussion

3.1. Preparation and characterization of diamond nanowires decorated with nickel nanoparticles (BDD NW/Ni NPs)

Fig. 1A shows the different steps involved in the construction of the immunosensor. Micrometer long BDD NWs were produced using a mask-less etching process of polycrystalline borondoped diamond thin films of 15 μm thickness by reactive ion etching with oxygen plasma as previously reported by us.²¹ Fig. 1B shows SEM images of the BDD interface before and after RIE for 180 min. While the polycrystalline facets stay clearly distinguishable, long needle-like shaped BDD NWs of 3 ± 0.2 μm length and a tip diameter of 30 ± 20 nm were formed using this etching protocol. The formed BDD NWs were then modified with Ni NPs by immersion into an aqueous solution of NiSO₄ (5 mM) in 0.01 M PBS (pH 6.5) and by application of electrochemical pulses. A representative scanning electron microscopy (SEM) image of the resulting BDD NW/Ni NP interface after cathodic deposition of Ni NPs at −1.3 V/Ag/AgCl using 10 potential pulses of 200 s followed by electrochemical cycling in 0.1 M NaOH to enrich the β-Ni(OH)₂ phase is shown in Fig. 1B. The particles are 20 ± 5 nm in diameter with a particle density of 150 Ni NPs per μm² for 200 s deposition time. This is somewhat larger than previously reported shorter BDD NWs.²⁴

The chemical composition of the interface was investigated using X-ray photoelectron spectroscopy (XPS). The Ni 2p XPS spectrum (Fig. 2A) shows two major peaks centered at 856.3 and 873.8 eV with a spin-energy separation of 17.5 eV, corresponding to Ni 2p₃/₂ and Ni 2p₁/₂, respectively. These bands are characteristic of the Ni(OH)₂ phase and in agreement with other literature reports.²⁵–²⁷ The two extra peaks centered at 861.8 and 879.2 eV are due to Ni 2p₃/₂ and Ni 2p₁/₂ satellite peaks. The atomic percentage of nickel present on the BDD NWs is 15.4 at%.

The oxidation state of the deposited nickel was confirmed by the presence of a quasi-reversible redox peak at约为0.47 V/Ag/AgCl in the cyclic voltammogram of the BDD NW/Ni NP electrodes in PBS (Fig. 2B) assigned to Ni(OH)₂/NiOOH. The hydrated nickel hydroxide could be present in two crystalline forms: the hydrated α-Ni(OH)₂ and the anhydrous β-Ni(OH)₂, the latter being the more stable and preferentially formed by cycling in NaOH (0.1 M).²⁸

3.2. Preparation of the electrochemical immunosensor

The BDD NW/Ni NP electrode was used for the construction of an electrochemical immunosensor. The strategy employed here for the immobilization of anti-IgG is based on the preferential coordination of biotinylated anti-IgG on the Ni NPs as suggested previously by Cosnier and co-workers using poly(pyrrole-nitrilo-triacetic acid)-Cu²⁺ films.²² The advantage of this approach would be the possibility of controlled immobilization of biotinylated antibodies and antigens with the omission of avidin layers, influencing the electrochemical behaviour of the electrical interface. The affinity of the biotin-tag to the Ni NPs is also believed to be weaker than the avidin–biotin interaction, allowing easy regeneration of the interface through a simple EDTA wash.

Electrochemical impedance spectroscopy was employed to probe the change in the interfacial properties upon binding of biotin-labeled anti-IgG to BDD NWs/Ni NPs. As the dynamics of charge transfer at electrode interfaces are strongly influenced by the nature of the electrode surface, immobilization of biomolecules is anticipated to alter the interfacial electron-transfer features. Fig. 3A shows the Nyquist plot for the BDD NWs/Ni NPs
before and after incubation with biotinylated anti-IgG (100 µM mL⁻¹) for 100 min using Fe(CN)₆⁴⁻/³⁻ as the redox probe. It was found that the capacitive changes were not as sensitive as electron transfer resistance ($R_{ct}$), presented by the real component Re($z$) of the impedance. The change in $R_{ct}$ is dependent on the amount of biotin-labeled anti-IgG immobilized on the surface (Fig. 3B). Incubation of the electrode in biotin-labeled anti-IgG (100 µg mL⁻¹) results in an increase of the charge transfer resistance from 3953 ± 95 Ω (BDD NWs/Ni NPs) to 5541 ± 78 Ω (BDD NWs/Ni NPs/anti-IgG) and thus a maximal change of $\Delta R_{ct} = 1588 ± 65$ Ω (Fig. 3B). To avoid the non-specific interaction, the interface was furthermore incubated in a BSA solution (100 µg mL⁻¹) for 100 min. Incubation in BSA resulted in a further increase of charge transfer resistance by 150 Ω.

In a control experiment, BBD NW electrodes without Ni NPs were incubated in an identical way in biotin-tagged anti-IgG (100 µg mL⁻¹) solution and then washed several times with PBS under ultrasonication. The recorded $\Delta R_{ct}$ was only $328 ± 40$ Ω, highlighting the importance of Ni NPs for a strong binding of biotin-labeled anti IgG.

### 3.3. Electrochemical detection of IgG binding by BDD NWs/Ni NPs/anti-IgG

The functional immunosensor was dipped into 0.01 M PBS (pH 7.4) containing various concentrations of IgG at room temperature for 40 min and then rinsed with PBS to remove any unbound analyte. The immune-reaction between surface linked anti-IgG and IgG in solution was monitored by EIS in the presence of Fe(CN)₆⁴⁻/³⁻ (0.01 M, PBS, pH = 7.4) as the redox probe. The change in the charge transfer resistance of IgG recognition in the range of 0–100 ng mL⁻¹ IgG is shown in Fig. 4. A linear relation between $\Delta R_{ct}$ and IgG concentration in the range of 0.3–400 ng mL⁻¹ was recorded with a correlation coefficient of $r = 0.9996$ according to $\Delta R_{ct} (k\Omega) = 0.02 + 0.0451 \times [\text{IgG}]$. The detection limit of IgG was determined to be $\approx 0.3$ ng mL⁻¹ from five blank noise signals (95% confident level). This detection limit is comparable to poly(o-aminobenzoic acid) modified...
planar BDD interfaces using alkaline phosphate as the label with the main advantage of a complete label-free detection in the case of BDD NWs/Ni NPs (Table 1). The detection limit of BDD NWs/Ni NPs is lower than that reported for carbon nanotube array, and gold-nanoparticle based sandwiched electrochemical immunosensors. It is, however, less sensitive than the sensor recently proposed by Kim and co-workers based on electrochemically reduced graphene oxide modified with an N-acryloxysuccinimide-activated amphiphilic polymer and functional polypyrrole modified interfaces. The reproducibility of the electrodes is expressed in terms of the relative standard deviation which is found to be 5.3% at an IgG concentration of 100 ng mL\(^{-1}\). The stability of the immunosensor was examined after 15 days storage in a refrigerator at 4 °C. The sensor retained about 97% of its initial sensitivity for 100 ng mL\(^{-1}\) IgG detection, indicating that the electrode has good stability.

The BDD NW/Ni NP interface could be regenerated in two different ways. Polyclonal IgG populations will typically have \(K_d\) values on the order of \(10^{-10}\) to \(10^{-7}\) M and can be disrupted upon treatment with 0.1 M NaOH. The Ni NP–biotin interaction should be weaker than biotin–streptavidin interactions (\(K_d = 10^{-15}\) M). The anti-IgG could thus be detached from the BDD NWs/Ni NPs by incubation in an aqueous solution of ethylenediaminetetraacetic acid (EDTA, 0.01 M) for 60 min and the BDD NWs/Ni NPs could be used for linking another antibody if desired.

### Table 1 Analytical performance of other electrochemical based immunosensors: poly-o-ABB: poly(o-aminobenzoic acid); poly(B.N.P.): copolymer carrying hydrophobic benzene ring, \(N\)-acryloxysuccinimide and poly(ethylene glycol) methacrylate, units; ALP: alkaline phosphatase, HRP: horseradish peroxidase; ANTA: \(N\)-a-bis(carboxymethyl)-L-lysine

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4. Conclusions

The development of an immunosensor based on a boron-doped diamond nanowire electrode was demonstrated. The immobilization of biotin-labeled bovine anti-IgG was accomplished through coordination of the biotin group to nickel nanoparticles, electrochemically deposited onto the BDD NW electrode. This immobilization was found to be specific towards the Ni NPs and proved to be as efficient as the conventional avidin–biotin system. The sensitivity toward the label-free detection of IgG is notably comparable to poly(o-aminobenzoic acid) modified planar BDD interfaces using alkaline phosphatase as the label. The immunosensor could be easily regenerated by incubation in a basic solution which results in the dissociation of the antigen–antibody complex. In addition, incubation in an aqueous solution of EDTA detached the biotin-labeled antibody from the Ni NPs and recovered the initial BDD NW/Ni NP interface. This will allow, in future, the immobilization of different anti-bodies using the same interface.

### Acknowledgements

R.B., Y.C. and S.S. gratefully acknowledge the financial support from the Centre National de Recherche Scientifique (CNRS), the Université Lille 1 and the Nord Pas de Calais region. Support from the European Union through a FP7-PEOPLE-IRSES (PHOTORELEASE) and FP7-PEOPLE-ITN (MATCON) are acknowledged. Many thanks to Mr Sobczak in obtaining the XPS data.

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